Micro X-Ray Fluorescence Computed Tomography and Radiography on Daphnia Magna

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Daphnia magna (Fig. 1) belongs to the order Cladocera and are members of a collection of animals that are broadly termed as “water fleas”. These small crustaceans (~1-4 mm) are being used to study the mechanism of intoxication by metals such as Zn and Ni and to develop so-called biotic ligand models (BLM’s). It has been shown that Daphnias respond differently to Zn-exposure when this occurs via the water and/or via the food (e.g. in terms of reproductive behaviour) [1]. In order to gain further insight in this difference of response to various exposure routes, it is important to study the 2D and 3D-elemental distributions, and more specifically of metals, within the organism subjected to various forms of exposure.

Synchrotron radiation (SR) based scanning-XRF and computed X-ray fluorescence tomography (XFCT) offer the possibility of 2D/3D non-destructive elemental imaging with microscopic resolution, which have trace-level detection limits for the metal-ions of interest and require minimal sample preparation in comparison with other elemental analysis and speciation techniques [2]. For the analyses described below a via water exposed (120 µg/L Zn, 1week) and an unexposed Daphnia Magna sample were used, dehydrated through an acetone-water series and dried in hexamethyldisilazene (HMDS) [3]. The samples were fixed on a capillary using a minimum amount of paraffin, and the capillary was mounted on a goniometer-head (see Fig.2 with the experimental set-up).

The 2-D elemental projection maps were recorded by conventional micro-XRF scanning, and elemental distributions within the cross-section through the egg region were obtained on both samples by XFCT. The tomographic data-sets were reconstructed using a backprojection algorithm from the generated sinograms. The measured elemental maps for a non-exposed sample are shown in Figs. 3(a) and 4(a,b), corresponding to experimental conditions of continuous scanning mode [4] using a step size of 20 µm and exposure time of 0.3s/pixel. The 20 keV monochromatic microbeam was obtained by W/Ni multilayer monochromator coupled with an ellipsoidal single bounce capillary for focussing.

Figure 1: Light microscope image of Daphnia magna.

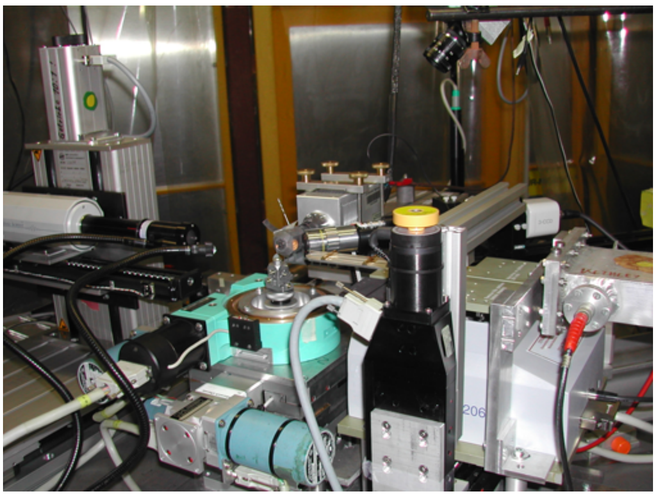

Figure 2: Experimental set-up at beamline L

Preliminary data analysis shows complementary regions of interest (ROI's) in various elements such as Zn, Fe, Ca and K as well as an elevated concentration of Zn in the water-exposed sample as compared to the non-exposed sample.

Absorption images of the non–exposed Daphnia sample were taken using a high resolution CCD-camera (0.8 µm/pixel) (see Fig.3b), which gives complementary information on the skeleton and inner structure and allows element-to-tissue correlation.

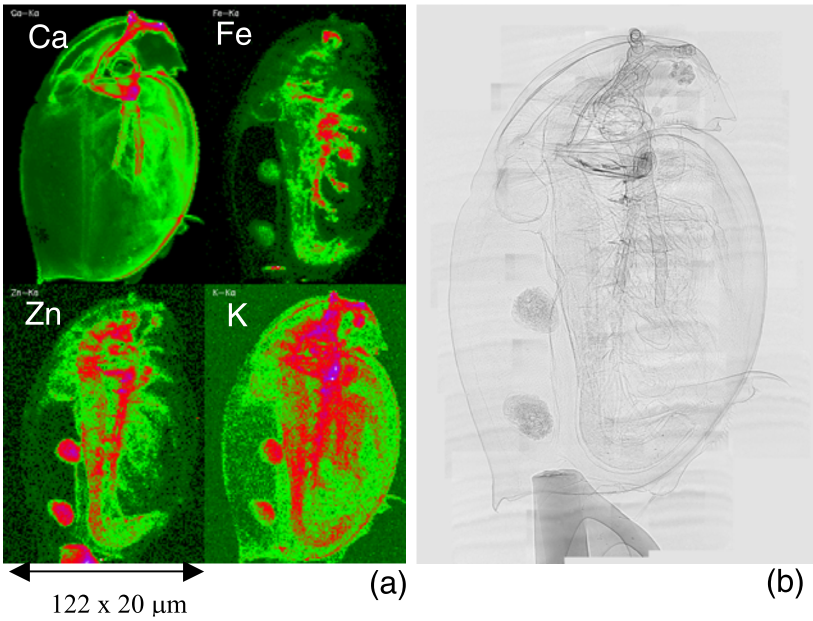

Figure 3: (a) 2D-element maps (Ca, Fe, Zn and K) and (b) composite radiographic image.

The tomographic reconstruction reveals the inner structure of the investigated Daphnia sample, demonstrating the possibility of localizing Zn-enriched microscopic regions, such as the eggs, shown in Fig.4(b). Further analysis will be performed to quantify these results and to study the organ/tissue-specific enrichment of Zn and other potentially toxic elements through metabolism.

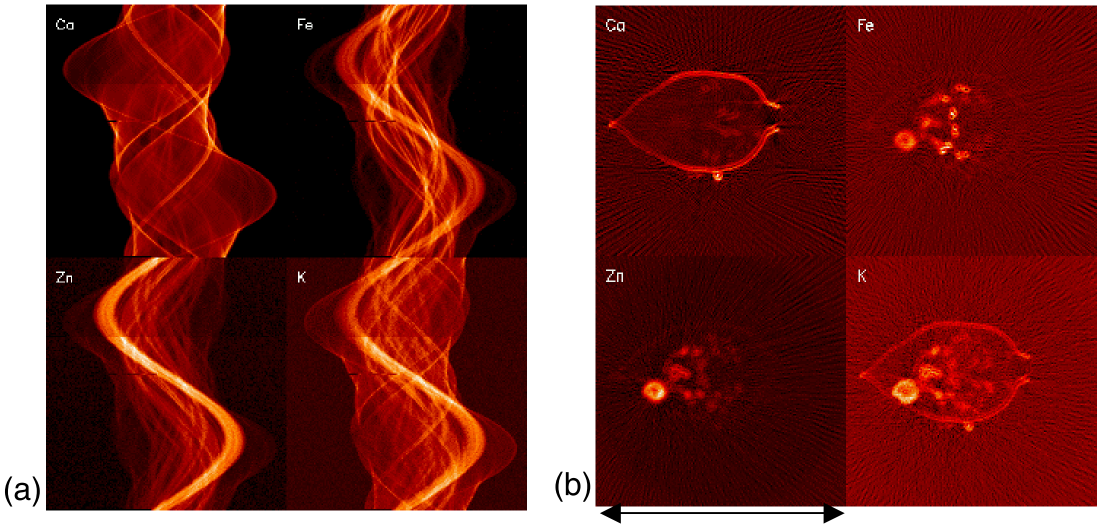

Figure 4: (a) Elemental sinograms and (b) reconstructed distributions of Ca, Fe, Zn, K.

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